STUDIES IN THE METABOLISM OF APPLES.

II. THE RESPIRATORY METABOLISM OF GRANNY SMITH APPLES OF COMMERCIAL MATURITY AFTER VARIOUS PERIODS OF COOL STORAGE.*

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(Six Text-figures.)

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Introduction.

In 1928 Blackman and Parija advanced the theory that two main factors, namely, "starvation drift" and "lowering of organization resistance", determine the respiratory drift of an apple, as measured by output of carbon dioxide. Onslow, Kidd and West (1931) suggested that the respiratory activity was limited by the supply of "active fructose" when the enzymic capacity of the cell was high in proportion to this supply, whereas respiratory activity was limited mainly by enzymic capacity when substrate supply was high.

Lowering of organization resistance and increase in enzymic capacity might bring about a rise in respiration rate, but could not account for the falling respiration rates observed during senescence. Blackman and Parija (1928) attributed the senescent decline to starvation drift. The investigations of Onslow, Kidd and West (1931) indicate that in some apples starvation limits respiration rate during senescence, but in a previous paper Kidd and West (1930) drew attention to certain data which could not be reconciled with the theory that starvation is always responsible for the senescent decline in respiration. It seems, then, that some factor other than starvation drift, lowering of organization resistance or increase in enzymic capacity must influence respiration rate during certain periods of the history of the fruit.

In the first paper of this series (Trout *et al.*, 1942) preliminary data were presented indicating the way in which changes in the composition of the internal atmosphere of an apple may be correlated with changes in its respiratory activity. The present paper is concerned with investigations on Granny Smith apples of commercial maturity of the 1941 and 1942 seasons, and it will be shown that the composition of the internal atmosphere probably plays a vital part in controlling the respiration rates of these apples during certain periods of their storage life.

Material and Methods.

The apples for the 1941 investigations were obtained from thinned trees at Orange, N.S.W. They were stored at 1°C. Samples of 5 fruits were withdrawn at intervals of 2 to 4 weeks and their subsequent behaviour was observed at 18·3°C. The first sample was taken approximately 3 weeks after the date of picking. The respiration rate, composition of the internal atmosphere, and colour of each fruit were observed at regular intervals. As soon as a fruit showed any signs of mould or other disorder it was discarded. For most samples the observations were continued for at least 4 weeks, but some of the later samples were in poor condition on removal from store and could not be kept for more than 1 or 2 weeks at 18·3°C. During 1941 the adequacy of the sample size was checked at frequent intervals by comparing duplicate samples.

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The fruits for the 1942 work were part of the normal commercial picking from Batlow, N.S.W. The investigations were carried out in the same way as those of the previous year, a sample having been withdrawn regularly every second week. The subsequent behaviour of the fruits was observed at 21°C. instead of 18·3°C. This was due to the fact that facilities for keeping the fruits at the lower temperature were not available. Colouring rates were not observed during 1942.

Respiration rates were measured by the Pettenkofer method and expressed in milligrams of carbon dioxide per 100 kilograms per hour.

Measurements of the concentrations of oxygen and carbon dioxide in the internal atmosphere were obtained by the method described in the first paper of this series (Trout *et al.*, 1942). The apples were fitted with detachable gas-sampling pipettes and were held in respiration vessels only during the periods necessary for respiration readings. This was done to reduce the possibility of any effect of prolonged enclosure of the fruits.

Observations of skin colour were made by comparison with the standards in the "Dictionary of Colour" (Maerz and Paul, 1930).

Experimental Results—A.

1941 season: *Fruits put into cool store on 17.iv.41, three days after picking.*

Table 1 shows date of removal from store (and insertion of gas-sampling pipettes), date of first observation and date of final observation for each of the ten samples taken.

Table 1.

Fruits of 1941 Season.

Sample Number.	Date of Removal from Store.	Date of First Observation.	Date of Final Observation.	Number of Weeks in Cool Store.
1	6.v.41	12.v.41	27.vi.41	3
2	6.vi.41	9.vi.41	30.vii.41	7½
3	8.vii.41	9.vii.41	28.viii.41	12
4	5.viii.41	6.viii.41	5.ix.41	16
5	2.ix.41	3.ix.41	23.x.41	20
6	23.ix.41	24.ix.41	29.x.41	23
7	14.x.41	15.x.41	3.xii.41	26
8	5.xi.41	6.xi.41	19.xi.41	30
9	17.xi.41	19.xi.41	2.xii.41	31
10	3.xii.41	4.xii.41	12.xii.41	33

In samples 3 to 10 inclusive, the first observations were made 24 hours after removal from store, but in samples 1 and 2 the first observations were not made until 6 and 3 days, respectively, had elapsed. This fact must be borne in mind when comparisons are being made between the early results for different samples.

Respiration Rate.—The mean respiration rates at 18·3°C. of the ten samples are shown in Figure 1. In samples 1 to 4 inclusive, the respiration rates were high initially and fell during the periods of observation, small fluctuations occurring from time to time. The decline in respiration rate was most marked during the first few days after removal from store. At any given time after removal from store the respiration rate of each successive sample was higher than that of the preceding sample at the corresponding time after removal from store.

In sample 5 the mean respiration rate was high 24 hours after removal from store and fell during the subsequent 6 days. During the following week a distinct rise and a subsequent fall were observed in the respiration rate, the maximum being attained on the tenth day after removal from store. No marked rise was observed in the respiration rate of sample 6, but it is possible that an increase occurred between the ninth and thirteenth days after removal from store, no observations having been made during that period. In sample 7 the mean respiration rate rose during the first seven days, the peak apparently being reached on the seventh day. During the next two weeks the respiration rate declined steadily. Both samples 8 and 10 remained free from breakdown for only one week at 18·3°C. The respiration rates of these samples rose throughout the periods of observation. Sample 9 behaved in a similar way to sample 7 during the first week, the highest observed respiration rate being attained on the sixth day after removal from store. Its initial respiration rate was much higher than that of sample 7.

Composition of Internal Atmosphere.—The values for the concentrations of oxygen and carbon dioxide in the internal atmospheres of all the samples are shown in Figure 2. The values are means for each sample. Samples 1 to 4 again behaved similarly, the

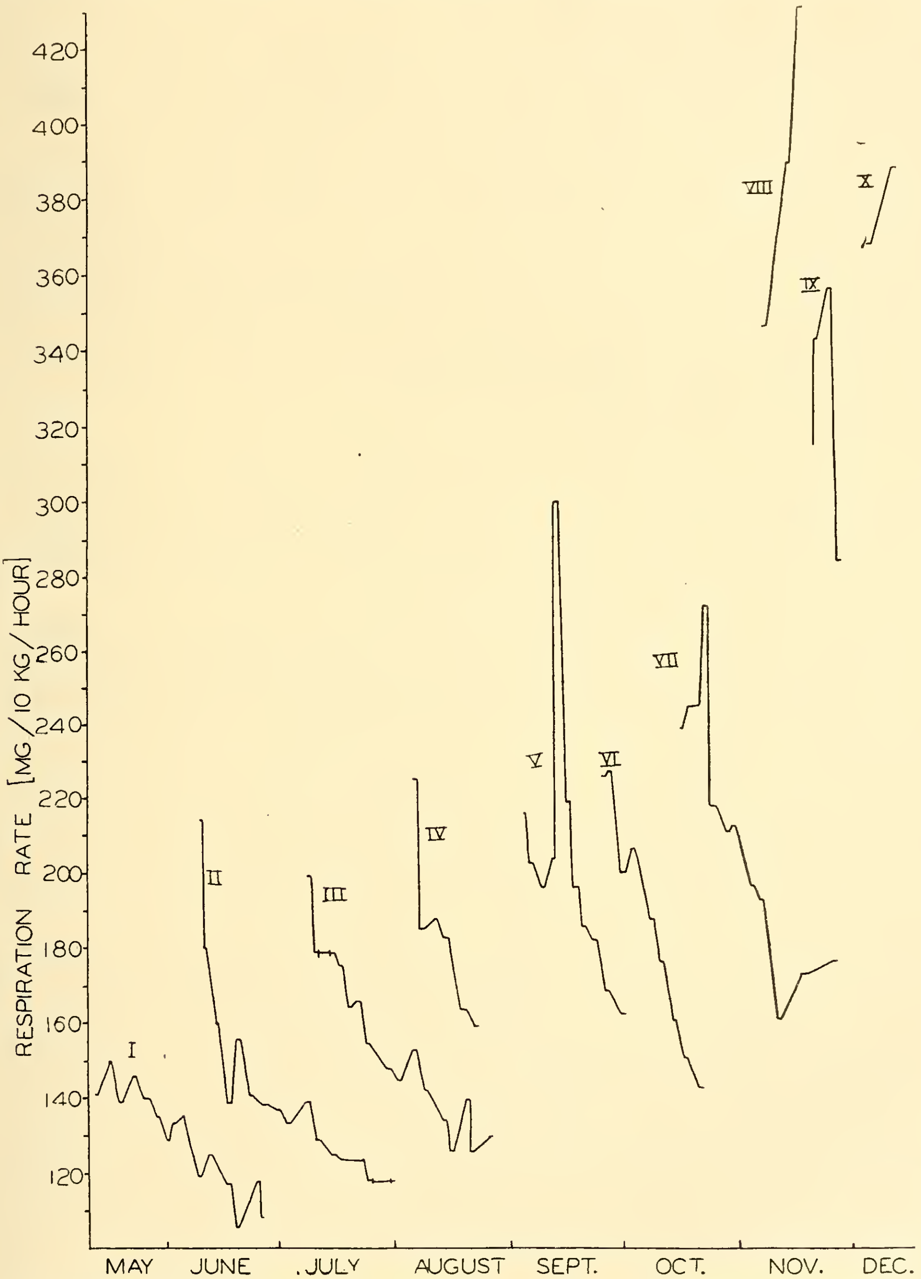

Fig. 1.—Mean respiration rates of 10 samples of Granny Smith apples taken during 1941. The order of removal from store is indicated by Roman numerals.

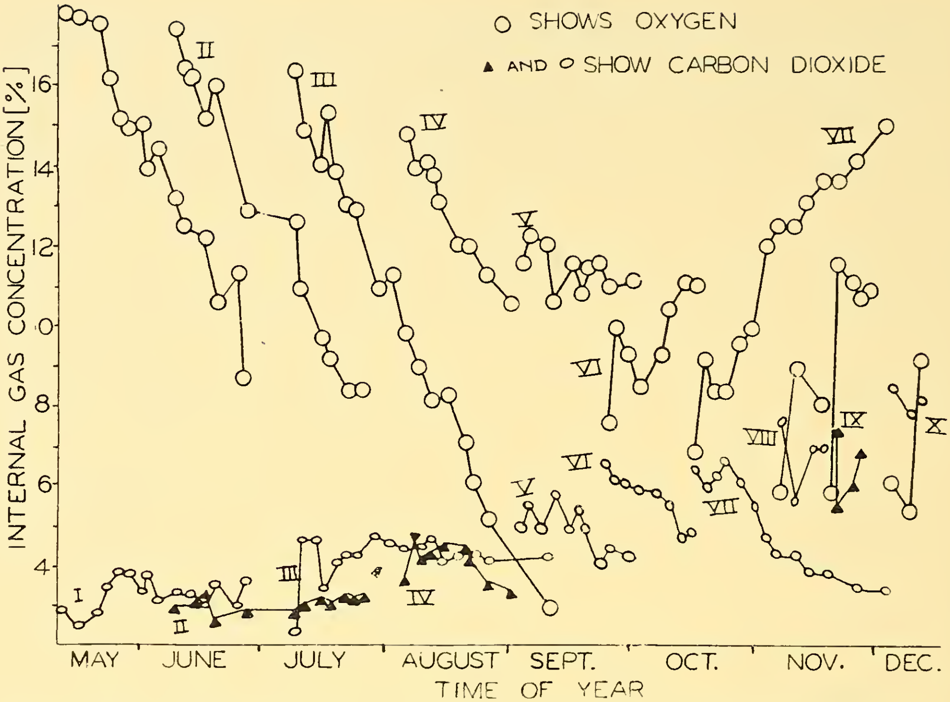

Fig. 2.—Mean concentrations of carbon dioxide and oxygen in the internal atmospheres of 10 samples of Granny Smith apples taken during 1941. The order of removal from store is indicated by Roman numerals.

internal oxygen concentrations being high initially and falling gradually with small fluctuations throughout the periods of observation. At any given time after removal from store the value for each sample was lower than that of the preceding sample at the corresponding time after removal from store. In sample 5 the initial internal oxygen concentration was lower than that of sample 4, but very little change occurred subsequently.

The internal oxygen concentrations of samples 6 and 7 were initially lower than those of any preceding sample, but rose in both samples after the first observation. This rise was followed in each instance by a slight decrease, after which the values increased steadily during the period of observation. The internal oxygen concentrations of samples 8, 9 and 10 were all very low initially and showed a tendency to rise with time.

The internal concentrations of carbon dioxide were constant in samples 1 and 2, except for a slight initial rise in sample 1. In sample 3 the concentration increased after the first observation to a value higher than those attained by previous samples, but did not change very much subsequently. In samples 5, 6 and 7 the internal concentrations of carbon dioxide were initially higher than those of preceding samples; but fell after the first week at 18·3°C. Later samples behaved in an irregular manner.

Resistance of the Skin to Gaseous Diffusion.—The resistances of the skin to the passage of oxygen (r_0) and of carbon dioxide (r_c) were calculated by the method used in the first paper of this series (Trout *et al.*, 1942). The mean resistances of the various samples are shown in Figure 3. In the first four samples r_0 increased steadily during the whole period of observation. The rate of increase was approximately the same for each sample (4 units in 30 days). The initial values in these samples were almost identical.

In sample 5, r_0 was initially higher than in the preceding samples, but the subsequent increase was only about one unit in 30 days.

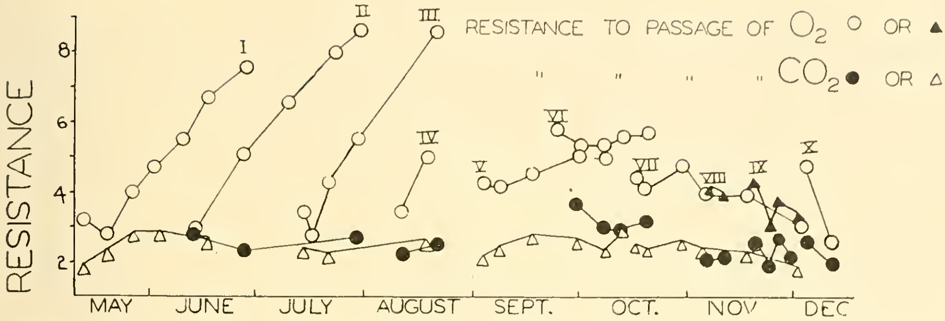

Fig. 3.—Mean resistances of the skin of 10 samples of Granny Smith apples of the 1941 season to the passage of oxygen and carbon dioxide. The order of removal from store is indicated by Roman numerals. For clearness, two symbols are used for both variables.

Sample 6 had the highest initial value for r_o but no subsequent increase occurred. In samples 7, 8, 9 and 10 the mean r_o decreased during the period of observation.

The values for r_c did not change very much. In sample 1 there was a slight initial increase. The r_c of sample 6 was higher than that of any other sample.

Colouring Rate.—There was no difference between the colouring rates of the various samples.

Experimental Results—B.

1942 season: *Fruits put into cool store on 5.v.42, two weeks after picking.*

Table 2 shows the dates of removal from store, insertion of gas-sampling pipettes, first observation and final observation for each of the fifteen samples taken.

Table 2.
Fruits of 1942 Season.

Sample Number.	Date of Removal from Store.	Date of Insertion of Sampling Pipette.	Date of First Observation.	Date of Final Observation.	Number of Weeks in Cool Store.
1	——	5.v.42	6.v.42	22.vi.42	0
2	11.v.42	12.v.42	14.v.42	13.vii.42	1
3	25.v.42	26.v.42	27.v.42	3.viii.42	3
4	8.vi.42	9.vi.42	10.vi.42	3.viii.42	5
5	22.vi.42	23.vi.42	24.vi.42	19.viii.42	7
6	7.vii.42	8.vii.42	9.vii.42	15.ix.42	9
7	20.vii.42	21.vii.42	22.vii.42	6.x.42	11
8	3.viii.42	4.viii.42	5.viii.42	22.x.42	13
9	19.viii.42	19.viii.42	20.viii.42	6.xi.42	15
10	2.ix.42	3.ix.42	4.ix.42	20.xi.42	17
11	22.ix.42	23.ix.42	24.ix.42	24.xi.42	20
12	12.x.42	12.x.42	13.x.42	13.xi.42	23
13	26.x.42	26.x.42	27.x.42	8.xii.42	25
14	10.xi.42	10.xi.42	11.xi.42	9.xii.42	27
15	24.xi.42	24.xi.42	25.xi.42	10.xii.42	29

Sample 1 had not been in cool store. In samples 3 to 8 inclusive, and samples 10 and 11, the gas-sampling pipettes were inserted the day after removal from store, and the first observations were made 24 hours later. In sample 9, and in samples 12 to 15 inclusive, the sampling pipettes were inserted on the day of removal from store and the first observations were made 24 hours later. The effects of wounding due to the insertion of the gas-sampling pipettes have been investigated and it has been shown that they are small and transient, being over within 24 hours.

Respiration Rate.—The mean respiration rates at 21°C. of the samples taken during 1942 are shown in Figure 4. It is clear that the first five samples behaved similarly. The mean respiration rate of each sample decreased by approximately 50% during the period of observation. Except in sample 5, where a small initial increase was observed, the rate of decrease was greatest during the first few days after removal from store. This was similar to the behaviour of the early samples of 1941. In the 1942 fruits there was no significant difference between the respiration rates of the first four samples on comparable dates after removal from store.

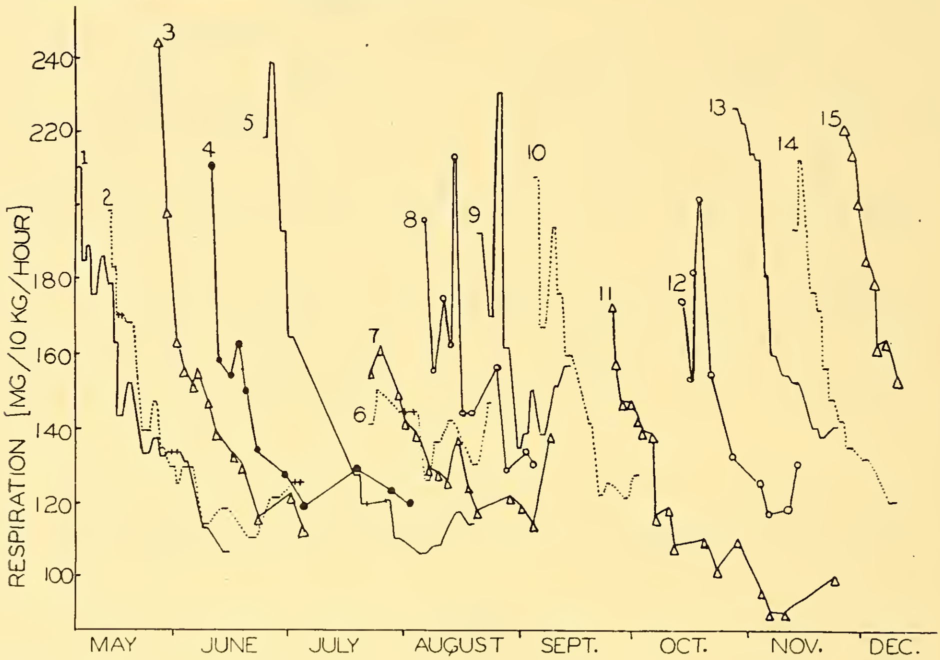

Fig. 4.—Mean respiration rates of 15 samples of Granny Smith apples taken during 1942. The order of removal from store is indicated by the serial numbers. Some of the curves have been curtailed (see text).

No measurements were obtained of the respiration rate of sample 6 until 14 days after removal from store. Subsequent to this period the rate fluctuated a good deal, but did not decrease significantly.

The initial respiration rate of sample 7 was much lower than those of preceding samples. It fell during the first 43 days of observation at a rate comparable with the rates of fall shown by preceding samples when they had been out of store for several days. During the last weeks of the period of observation, the respiration rate rose irregularly. This was probably associated with the onset of death of the tissue, as by this time the fruits were in poor condition.

Samples 8, 9, 10 and the only fruit of sample 12 which remained free from mould attack, behaved similarly. The mean respiration rates decreased initially and then rose to a peak. After this peak was passed the respiration rate decreased again, often fluctuating markedly. The behaviour of samples 8, 9 and 10 during the last 50 days of observation is not shown in the figure. In all three samples the respiration rates showed no major changes during this period.

The respiration rate of sample 11 was comparatively low initially and fell steadily during the period of observation. In no fruit of this sample was there any suggestion of the very great fluctuations observed in the three samples immediately preceding it.

The mean respiration rates of samples 13 and 15 were initially very high and subsequently fell slowly. No fluctuations were observed.

In sample 14 a marked rise occurred in the respiration rate of one fruit 4 days after removal from store. Small increases were observed in two fruits. The remaining two fruits showed no increase. During the subsequent weeks the respiration rates of all fruits decreased steadily.

Composition of the Internal Atmosphere.—The mean concentrations of oxygen present in the internal atmospheres of all the samples and the mean concentrations of carbon dioxide for three of the samples are shown in Figure 5. The first five samples behaved similarly to the early samples of 1941. From the time of removal from store the internal oxygen concentration drifted downwards, the rate of fall being approximately the same in all samples. There were indications that the concentration might have been rising in sample 4 at the end of the period of observation. Fluctuations were not

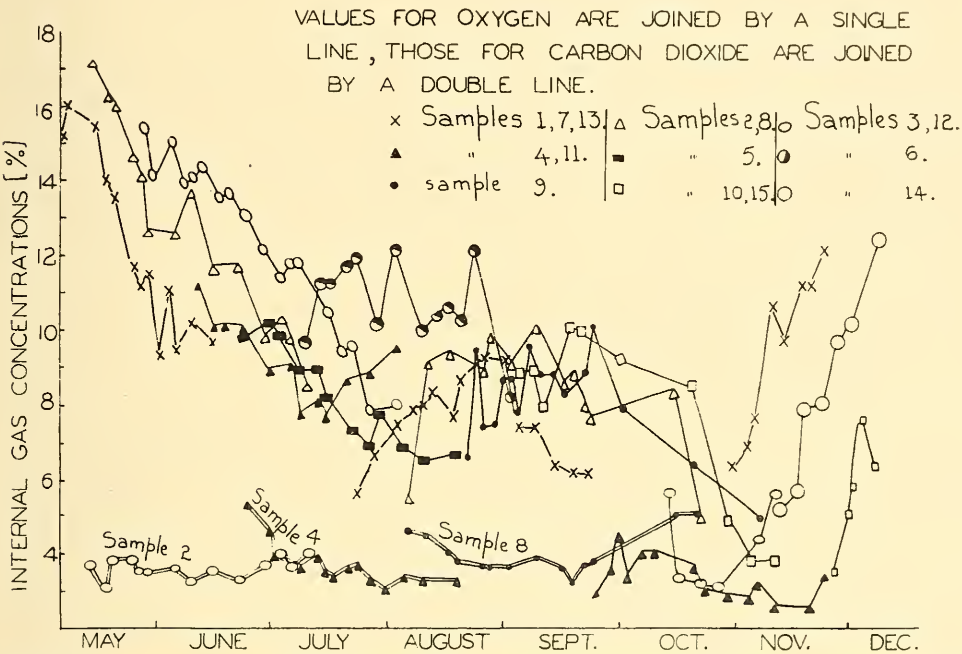

Fig. 5.—Mean concentrations of oxygen in the internal atmospheres of 15 samples of Granny Smith apples taken during 1942. The corresponding concentrations of carbon dioxide are indicated for 3 samples only.

great in these early samples. The mean initial internal oxygen concentration was lower for each successive sample from 2 to 5 inclusive. In the first five samples the mean internal carbon dioxide concentrations either remained steady or fell gradually during the period of observation.

The mean initial internal oxygen concentration of sample 6 was equal to that of sample 5. During the period of observation large fluctuations occurred, but no definite trend was evident. In this sample the mean internal carbon dioxide concentration fell slowly during the first fortnight at 21°C.

In samples 7, 8 and 9 the internal oxygen concentration was initially very low, but rose subsequently, this rise being most sustained in sample 7, where it continued for about 4 weeks. Towards the end of the period of observation the mean internal oxygen concentration of each sample reverted towards the original value, that of sample 9 falling below its initial value. The mean internal carbon dioxide concentrations for these samples fell slowly with time, reaching their minimum values at approximately the same times as the corresponding internal oxygen concentrations reached their maxima. They subsequently increased and in samples 8 and 9 regained their original values.

Samples 11 and 12 had very low mean internal oxygen concentrations associated with carbon dioxide concentrations comparable with those of previous samples. Fluctuations occurred in both oxygen and carbon dioxide values, but no definite trends were evident.

In samples 13, 14 and 15 the mean internal oxygen concentrations were initially very low, but increased with time at 21°C. The corresponding internal carbon dioxide concentrations were relatively high initially, but decreased with time.

Resistance of the Skin to Gaseous Diffusion.—The mean resistance of the skin to diffusion of oxygen (r_o) increased in samples 1 to 12 inclusive, the rate of increase being very great (sometimes as high as 6 units in 30 days) in all samples except 7 (see Figure 6). In sample 7 the mean r_o, although initially higher than that of any other sample except 11, remained constant for the first 30 days at 21°C., and rose slightly at the end of the period of observation.

In samples 13, 14 and 15 there was very little change in mean r_o.

During the first 5 months there was a tendency for the initial mean r_o on removal from store to increase, but large fluctuations occurred between samples (see Figure 6). Samples 7 and 11 had the highest initial values for r_o. The initial value of sample 6 was not obtained.

The mean resistance of the skin to the diffusion of carbon dioxide (r_c) usually increased during the period of observation (see Figure 6). No increase was observed in sample 7.

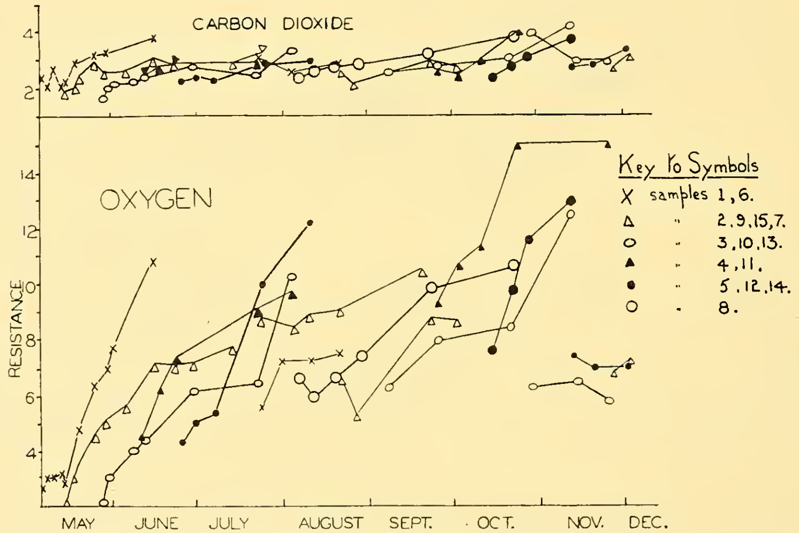

Fig. 6.—Mean resistances of the skin of 15 samples of Granny Smith apples, taken during 1942, to the passage of oxygen and carbon dioxide. Some of the curves for carbon dioxide have been curtailed. This has only been done where no further change occurred.

The fluctuations observed in the initial respiration rate, initial internal oxygen concentration and initial r_o were not due to inadequacy of the five-fruit sample. Although no check samples were run for comparison during 1942, the variability between fruits within each sample was not great. Had the sample size been inadequate, far more variability would have been observed within samples. The variability observed between samples must, therefore, have been due to some factor which was not controlled adequately during 1942. For some of the 1942 samples (2 to 8 inclusive and 10 and 11) a longer time elapsed between removal from store and placing at 21°C. than for the other 1942 samples or the 1941 samples. During this period, which was sometimes as long as 24 hours, the temperature was not controlled. However, it is very doubtful whether the variability between samples could be explained by such small differences in treatment.

Discussion of Results.

Comparison of Results for 1941 and 1942.—The respiratory behaviour of the 1941 fruits and that of the 1942 fruits was similar in some respects. The respiration rates of early samples in both seasons were high shortly after removal from store, and declined throughout the first 3 or 4 weeks at the higher temperature. The rate of decline was fastest during the first few days. In 1941 the type of respiratory curve did not change until about 4 months after picking, but in 1942 the change was evident after 3 months. Respiration curves for later samples of both seasons showed a tendency to rise shortly after removal from store. This rise was of short duration and was followed by a decline, except when the apples deteriorated so quickly at the high temperature that curves of long duration could not be obtained. For some unknown reason the respiration rates of the later samples of the 1942 season fluctuated much more than those of later samples of the previous season.

A gradual increase in respiration rate from sample to sample was observed during 1941, but was not evident in the 1942 samples.

The compositions of the internal atmospheres of fruits of the two seasons were also somewhat similar. In early samples of both seasons the internal oxygen concentration fell throughout the period of observation. In the latest group of samples the internal oxygen concentration was low initially, but rose during the period of observation. Between these extremes there were six samples in 1942 and only one sample in 1941 in which the internal oxygen concentration fluctuated a good deal, but neither increased nor decreased continually.

During both years there was a strong tendency for initial internal oxygen concentration to decrease from sample to sample.

The internal carbon dioxide concentrations of early samples of both seasons either increased with time or remained steady. Those of later samples frequently decreased with time. In 1941, there was a tendency for the initial value to increase with successive samples, but this was not evident in 1942.

In early samples of both seasons the changes in the resistance of the skin to gaseous diffusion were somewhat similar. The resistance to the diffusion of oxygen (r_o) was comparatively low initially in samples 1 to 4 of the 1941 season and samples 1 to 3 of the 1942 season. It increased very swiftly at the higher temperature, the ultimate values attained by the 1942 samples being very much higher than those attained by the 1941 fruits. In 1941 samples which had been stored for more than 4½ months, the r_o increased very little or remained constant at the higher temperature, and samples which had been stored for 6 months or more apparently decreased in r_o. In 1942, samples which had been stored for 5½ months still increased in r_o when placed at 21°C. Samples which had been stored for 6 months or more showed very little change in r_o during the periods of observation. Some individuals in these samples rose slightly in r_o, some decreased slightly and others remained steady.

In samples of both seasons there was a tendency for the initial r_o to increase from sample to sample, the increase being much greater in 1942 than in 1941. This tendency disappeared towards the end of the year.

Changes in r_c were less marked than those in r_o. In early samples of both seasons r_c either increased or remained steady. In later samples of the 1941 season, but not of the 1942 season, there was a tendency for r_c to decrease.

The final samples of the latter season were in far better condition on removal from store than those of 1941 and could therefore be studied at the higher temperature for longer periods. The decrease in resistance observed in the later samples of 1941 might have been due to some kind of deterioration of the skin, as several of the fruits showed superficial browning. It must be remembered that the values for r_o were calculated using the assumption that the respiratory quotient of the fruits was constant throughout the period of experimentation. If the respiratory quotient changed, the values given would be untrue. However, preliminary work done on respiratory quotients during the later months of 1942 indicates that the respiratory quotient remains approximately equal to 1·0.

Existing Theories of Respiratory Drift.—In recent years several workers have pointed out that the changes in metabolic activity of fruits can not be fully attributed to the interaction of "lowering of organization resistance" and "starvation drift" suggested by Blackman and Parija (1928).

The reasons for the "climacteric rise" and "senescent decline" in respiration are still unknown. Kidd and West (1930) have pointed out that in apples the climacteric rise does not appear to be associated with any corresponding change in the concentration of any of the estimated carbohydrate constituents of the cells. They put forward the hypothesis that the rise is due to some "protoplasmic factor". Later experiments by the same workers (1933) indicate that the onset of the climacteric in apples is accelerated by concentrations of oxygen greater than 21%. Low concentrations of oxygen not only delay the onset of the climacteric but decrease its magnitude. Pre-climacteric apples will not go through a climacteric phase if kept in the absence of oxygen.

The starvation theory of Blackman and Parija (1928) will not always explain the senescent decline. Kidd and West (1930), working with apples, and Gustafson (1929), working with tomatoes, have shown that post-climacteric fruits may contain an abundance of the supposed substrates of respiration. Wardlaw and Leonard (1936), working with tropical fruits, have suggested that the reduction of internal oxygen supply, which occurs in many fruits during senescence, may be "a causal factor which explains the running down of the metabolic system, even though substrates for respiration are abundantly present".

Blackman and Parija did not consider the importance of oxygen as a limiting factor in the respiratory decline of some of their fruits, although they actually recorded a marked rise in respiration rate, which occurred when a senescent apple was transferred from air to pure oxygen, and a marked fall, which occurred when another fruit was transferred from air to 5% oxygen (papers I and II of the Blackman-Parija series). Moreover, for the apple in pure oxygen (paper II, p. 486), the respiration curve after equilibrium had been reached was exactly parallel to the declining "air-line respiration" curve. If substrate had been governing the rate of fall of the air-line respiration, the oxygen respiration curve should have fallen more steeply than the air-line respiration curve, since substrate was presumably being used faster. In discussing the effect of altering the oxygen concentration around the fruit, Blackman suggested that the production of the substrate for glycolysis was influenced by the rate of an oxygen-activated reaction, and that the rate of glycolysis was affected by the amount of substrate available. Thus, according to Blackman's own theory, oxygen played a vital part and it was apparently not the amount of reserve carbohydrate present but the availability of that reserve which determined the rate of respiration. In other words, oxygen supply limited respiration when the fruit was in air, whether by the means suggested by Blackman or in some other way.

It is interesting to note that when Kidd and West (1934) obtained measurements of the porosity (the inverse of r_o or r_c) of Bramley's Seedling apples, the values were apparently constant up to the time of the climacteric rise, but gradually decreased during senescence. This seems to support the theory that oxygen supply may become a limiting factor during senescence. Blackman and Parija probably realized that oxygen supply could be used artificially as a limiting factor, but, having no information about the natural changes which may occur in the permeability of the fruit, did not suspect that some apples under normal conditions might suffer from lack of oxygen.

Relation Between Internal Oxygen Concentration and Respiration Rate.—It has already been shown in the first paper of this series (Trout *et al.*, 1942) that there was a strong positive correlation between internal oxygen concentration and respiration rate in some Granny Smith apples of the 1940 season. The data obtained during 1941 and 1942 have been examined with a view to discovering more about this relationship.

In each of the first four samples of the 1941 season there was a strong positive correlation between internal oxygen concentration and respiration rate. For samples 1, 2 and 3 the regression coefficients were 4·8, 6·89 and 6·37 respectively, and were highly significant ($P << 0{\cdot}001$), but not significantly different from each other. For sample 4 the regression coefficient was 21·06, and this was significantly different from those of previous samples ($P << 0{\cdot}001$). In sample 5 there was no correlation between internal oxygen concentration and respiration rate. In samples 6 and 7 the correlation was again strong, but negative. The regression coefficients were −17·39 and −11·61 respectively, and were significant ($P < 0{\cdot}01$). The difference between them was not significant. Later samples did not last long enough for correlations to be observed.

Similar correlations have been found in the data for 1942. In the first five samples a strong positive correlation between internal oxygen concentration and respiration rate was evident. Early observations on sample 6 were not made, so it is not known whether there was any correlation in this sample. In the next six samples (7 to 12 inclusive) there was no correlation when each sample was considered as a whole. Certain individual fruits in these samples showed negative correlations. In some fruits a negative correlation was evident during the first weeks at 21°C., but broke down later. In the final samples (after sample 12) a strong negative correlation was observed.

Thus in both 1941 and 1942, there was a definite positive correlation between internal oxygen concentration and respiration rate in samples removed from store early in the season, and a definite negative correlation in those removed from store late in the season. In fruits removed on an intermediate date there was generally no apparent correlation between internal oxygen concentration and respiration rate. These facts are in accordance with the theory that early in the season oxygen supply is an important factor limiting respiration rate, whereas late in the season respiration rate is governed by some other factor, and internal oxygen concentration is governed by respiration rate.

The internal oxygen concentration must be determined by the rate of utilization and the rate of diffusion through the skin. In the early samples of both seasons the resistance of the skin to the passage of oxygen (r_o) rose steeply at the high temperature, and the internal oxygen concentration and respiration rate both fell. This is in accordance with the theory that respiration rate is limited by oxygen supply at this stage. In the final samples r_o either did not change very much or fell. Respiration rate, apparently limited at that stage by some factor other than oxygen supply, fell during the period of observation. The internal oxygen concentration rose as the rate of utilization decreased.

In the intermediate samples mean respiration rate was not correlated with oxygen supply. The increase in r_o which occurred in these samples apparently prevented the internal oxygen concentration from rising even though the rate of utilization fell considerably. In these samples the few individual apples which showed a negative correlation between internal oxygen concentration and respiration rate were those in which r_o did not change. In fruits which showed a negative correlation for a short time after removal from store, the r_o did not begin to increase immediately.

Thus it seems that oxygen supply, governed by the resistance to diffusion of oxygen, plays a very important part in the respiratory metabolism of fruits removed from store early in the year. In fruits removed later in the year, respiration is limited by some other factor, possibly starvation and progressive death of the tissue, the apples being in poor condition at the end of the period of observation.

In early samples the resistance of the skin to the diffusion of oxygen increased very rapidly after removal from store. The rise in initial values suggests that the increase also took place in store, but at a much slower rate. The reason for the increase is not definitely known. The Granny Smith apple develops a very noticeable oily coating on the skin. In early samples this had not developed at the time of removal, but it developed at the high temperature. In the later samples the fruits were greasy on removal from store. The increasing resistance to gaseous diffusion may be connected in some way with the development of this natural oil.

The development of natural greasiness has been observed in most varieties of apples. During 1942 the writer observed it in Delicious apples, where it was accompanied by falling internal oxygen concentrations. Delicious apples were observed after various periods of cool storage. The early samples became greasy at the high temperature, but later samples did not. In these later samples the resistance to gaseous diffusion did not increase very much.

Markley and Sando (1931) investigated the natural wax-like coatings of several varieties. They found that they consist of two fractions—one soluble and the other insoluble in low-boiling petroleum ether. The former is oily and consists mainly of triacontane; the latter is dry and powdery and has been given the name "ursolic acid". As apples mature the oily fraction increases faster than the powdery fraction. Markley and Sando are of the opinion that the change in ratio between the two fractions is accompanied by a change in physical properties, which may affect permeability to gases.

In later investigations (1933) the same workers found that the quantities of cutin present in the cuticle are greater at maturity and at the end of the storage period than in the early stages.

During 1941, experiments were carried out by Dr. F. E. Huelin, Council for Scientific and Industrial Research, Homebush, on the natural waxy coating of Granny Smith apples (data unpublished). Fractions similar to those of Markley and Sando were isolated.

The Respiratory Drift in Granny Smith Apples.—The shapes of the respiration curves obtained for Granny Smith apples are puzzling when compared with the curves obtained by other workers for other varieties. According to many authors, including Blackman and Parija (1928), Kidd and West (1930), and Trout, Tindale and Huelin (1940), the respiration curve for ripening apples is characterized by a rise to a climacteric peak, more or less coincident with the onset of colouring, followed by a decline. This type of respiration curve has been observed in many other fruits—e.g., papaws (Wardlaw and Leonard, 1936) and bananas (Wardlaw, Leonard and Barnell, 1939).

It has already been shown in the first paper of this series (Trout *et al.*, 1942) that in Granny Smith apples the development of colour may occur independently of other ripening changes. During the three seasons (1940 to 1942 inclusive) during which the writer has recorded respiration curves for Granny Smith apples, no rise has been observed which could with certainty be called a climacteric rise. The peaks in the respiration curves of fruits removed from store late in 1941 and 1942 (see Figures 1 and 4) were not usually pronounced enough in magnitude or duration to be compared with the climacteric peaks recorded by other workers. They were probably post-climacteric peaks comparable with those shown by Wardlaw and Leonard (1936) for papaws far advanced in senescence, or those shown by Krotkov (1941) for McIntosh apples.

During 1941 the respiration rates on comparable dates after removal from store increased from sample to sample. It is possible that the respiration rates of these apples were rising to a climacteric peak in store and that the peak was masked by the temperature effect on removal from store. Although fruits of so-called commercial maturity were used during both years, those of 1942 were initially more mature in appearance and flavour than those of 1941. This was probably due to the fact that a longer period elapsed betweeen the dates of picking and placing in cool store in 1942.

There are three possibilities regarding the occurrence of the climacteric rise in Granny Smith apples. These fruits may differ from other varieties in that no climacteric rise occurs. On the other hand, the peaks observed by the writer might have been climacteric peaks in spite of their dissimilarity to those recorded by other workers for other varieties. The third possibility is that the climacteric phase was over by the time the investigations were carried out. It might have occurred before the fruits were picked or it might have been occurring slowly in store and been obscured by the temperature effect on removal.

The first two possibilities are not very likely. There is a strong possibility that the fruits were in the post-climacteric condition during the whole period of observation after removal from store.

Kidd and West (1934) showed that the permeability of the Bramley's Seedling apple to oxygen and carbon dioxide did not change until after the climacteric rise. The data of Wardlaw and Leonard (1936, fig. 15, p. 648) for papaws and those of Wardlaw, Leonard and Barnell (1939, fig. 6) for bananas have been used by the writer to calculate the resistances of these fruits to gaseous diffusion (see Tables 3 and 3A).

In the papaw the internal carbon dioxide and oxygen concentrations were apparently governed by the respiration rate during the pre-climacteric phase, oxygen rising and carbon dioxide falling as respiration rate decreased. In both bananas and papaws the resistances to oxygen and carbon dioxide were more or less steady during the pre-climacteric phase and increased very much during senescence. If the Granny Smith

TABLE 3.
(From Data of Wardlaw and Leonard for Papaws.)

Time. (Arbitrary Units.)		Internal CO_2 (%).	Internal O_2 (%).	Respiration. (mg./10 Kg./hr.)	r_c	r_0
	0	7·0	14·0	2,900	0·24	0·24
Pre-climacteric.	3·0	3·5	17·5	1,530	0·23	0·23
	5·5	1·5	19·5	350	0·43	0·43
Climacteric.	6·5	3·0	15·0	1,400	0·22	0·32
Post-climacteric.	7·5	8·5	11·5	500	1·7	1·87
	8·5	14·0	5·0	1,400	1·0	1·11

TABLE 3A.
(From Data of Wardlaw, Leonard and Barnell for Bananas.)

Time. (Days.)		Internal CO_2	Internal O_2	Respiration.	r_c	r_0
Pre-climacteric.	1·0	2·0	14·0	500	0·4	1·2
	5·0	2·0	12·0	500	0·4	1·52
Climacteric.	6·0	11·0	1·0	2,500	0·44	0·73
Post-climacteric.	6·3	7·0	10·0	2,200	0·32	0·47
	7·0	8·0	13·0	1,500	0·53	0·55
	10·0	14·0	6·0	1,300	1·08	1·14
	13·0	20·0	2·0	800	2·5	2·4

apple behaves similarly to these fruits, then the increase in the resistance to gaseous diffusion in early samples is in accordance with the idea that the fruits were already senescent at the time of the first observation. Investigations being carried out at present on immature apples at various stages of development may throw some light on the problem.

Wardlaw and Leonard (1936) found that in extremely senescent papaws the internal oxygen concentration fell to zero, so that the fruit became anaerobic. The lowest internal oxygen concentration observed in normal Granny Smith apples was 0·9% in one of the fruits of the last sample taken in 1942. It is possible that some, at least, of the respiration was anaerobic when the internal oxygen concentration was very low.

Late in 1941, alcohol analyses on Granny Smith apples were carried out by Dr. F. E. Huelin, Council for Scientific and Industrial Research, Homebush. The technique used was that of Fidler (1931). A sample was taken, comparable with sample 5 used by the writer, in which the mean internal oxygen concentration was approximately 10%. After six weeks at 18·3°C. the percentage of alcohol in these fruits had not changed significantly from the initial value on removal from store (0·003%). Samples comparable with samples 6 and 7 used by the writer also showed no increase in alcohol concentration after removal from store. The internal oxygen concentrations of the 1941 samples did not fall as low as did those of the 1942 samples (see Figures 2 and 5).

In 1942 samples which were duplicates of some of those used in the measurements of respiration rate were used by the writer for alcohol analyses. Unless otherwise stated, alcohol analyses were carried out only during the first week after removal from store. In a sample comparable with sample 3, where the mean internal oxygen concentration was approximately 14%, the alcohol concentration was of the same order as that obtained by Dr. Huelin the previous year (0·004%). In a sample taken two weeks later, where the mean internal oxygen concentration was approximately 11%, it had risen to 0·009%. In later samples it was generally about 0·02%, but for those corresponding to samples 7 and 11, it was much higher (0·068% and 0·075% respectively). In the sample corresponding to sample 11, two analyses were carried out—one after 3 days at 21·1°C., the other after 10 days. The first analysis gave 0·075% of alcohol, the second gave 0·1102%. Samples 7 and 11 were those which had the lowest mean initial oxygen concentrations and the highest values for mean initial r_0 (see Figures 5 and 6).

On the assumption that alcohol formation is an index of anaerobic respiration, it seems that, under certain conditions, normal Granny Smith apples may carry out some anaerobic respiration due to oxygen limitation occurring naturally even when the fruit is in air. This agrees with the suggestions of Gustafson (1930, 1934). The amount of anaerobic respiration is probably very small, however, as preliminary observations carried out late in 1942 indicate that even when the initial oxygen concentration is as low as 3%, the respiratory quotient is approximately 1·0.

Summary.

For two years in succession investigations have been carried out on Granny Smith apples of commercial maturity after various periods of storage at 1°C. Observations have been made on respiration rates, concentrations of oxygen and carbon dioxide in the internal atmospheres, colouring rates and alcohol contents. Resistances of the skin to the passage of oxygen and carbon dioxide have been calculated from the respiration rates and the internal gas concentrations.

In apples which had been stored less than 3 or 4 months the respiration rates declined steadily over the periods of observation. The declining respiration rates were associated with decreasing internal oxygen concentrations and increasing resistances to the diffusion of oxygen. Marked changes in internal carbon dioxide concentrations and resistances to the diffusion of carbon dioxide were not generally observed. At this stage of the life of the fruit the respiration rate was apparently governed by the internal oxygen concentration.

In apples which had been stored for more than 5 months the respiration rates frequently showed marked fluctuations and generally exhibited downward trends. The internal oxygen concentrations either fluctuated irregularly or rose as the respiration rates fell. The resistance to diffusion of oxygen generally rose, but in some of the very late samples it remained steady or decreased. At this stage the internal oxygen concentrations were probably determined by two factors—respiration rate and the rate of increase of the resistance of the skin to the passage of oxygen. When resistance did not increase, falling respiration rates resulted in rising internal oxygen concentrations, but when resistance was increasing this effect was masked.

In some of the late samples the internal carbon dioxide concentrations decreased as respiration rates fell, but this effect, like the effect on internal oxygen concentration, was frequently masked by increasing resistance to gaseous diffusion.

There was no difference in the colouring rates after various periods of storage.

Alcohol analyses suggest that a small amount of anaerobic respiration occurs naturally when the internal oxygen concentration is low.

The relationship of these results to those obtained by other workers is discussed in detail.

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